

1.2. Differentiation of iPSCs into neural progenitor cells (NPCs)

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An abbreviated version of this protocol was published in Science in Jul 2020
Allele-specific open chromatin in human iPSC neurons elucidates functional disease variants
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Detailed protocol

Differentiation of iPSCs into neural progenitor cells (NPCs)

iPSCs maintained in mTeSR Plus media with routine removal of differentiated cells

Day -1: Passage iPSCs using ReLeSR following vendor's protocol;

Day 1: Refresh media using PSC Neural Induction Medium;

Day 2: Refresh media using PSC Neural Induction Medium;

Day 3: Refresh media using PSC Neural Induction Medium;

Day 4: Refresh media using PSC Neural Induction Medium. Check cells and remove non-neuronal cells;

Day 5: Refresh media using PSC Neural Induction Medium. Check cells and remove non-neuronal cells;

Day 6: Refresh media using PSC Neural Induction Medium. Check cells and remove non-neuronal cells;

Day 7: Refresh media using PSC Neural Induction Medium. Check cells and remove non-neuronal cells;

Day 8: Refresh media using PSC Neural Induction Medium. Check cells and remove non-neuronal cells;

Day 9: Refresh media using PSC Neural Induction Medium. Check cells and remove non-neuronal cells;

Day 10: Passage P0 NPCs using Rosette Selection Reagents following vendor's protocol. NPCs will detach after treatment of Rosette Selection Reagents. Collect and replat NPCs in Neural Expansion Medium with 5 μ M ROCK inhibitor in new Matrigel-coated dishes;

Day 11: Withdraw ROCK inhibitor and refresh media using Neural Expansion Medium;

Day 12-: NPCs should be maintained in Neural Expansion Medium with media refreshed every other day. Passage NPCs at 1:4 – 1:6 when reach 100% confluence.

5 μ M ROCK inhibitor should be supplemented for passaging and withdrawn 24 hr post passaging.

From P0-P3, NPCs should be passaged using Rosette Selection Reagents to remove non-neuronal cells; from P4, NPCs can be passaged using Accutase.

NPCs can be maintained for **no more than** ten passages.

Refer to vendor's manual (<https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2Fassets->

[Assets%2Fmanuals%2FMAN0008031.pdf&title=UHVjdG9jb2w6IEluZHVjdGlvbiBvZiBOU0NzIFVzaW5nIEdpYmNvIE5ldXJhbCBJbmR1Y3Rpb24gTV](https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2Fassets-Assets%2Fmanuals%2FMAN0008031.pdf&title=UHVjdG9jb2w6IEluZHVjdGlvbiBvZiBOU0NzIFVzaW5nIEdpYmNvIE5ldXJhbCBJbmR1Y3Rpb24gTV)) for non-neuronal impurity cells morphology.

iPSCs and NPCs are maintained in Matrigel coated tissue culture dishes.

Reagent list:

mTeSR Plus (StemCell# 100-0276)

ReLeSR (StemCell# 05872)

PSC Neural Induction Medium (ThermoFisher# A1647801): 490 ml Basal medium + 10 ml 50x supplement

Neural Expansion Medium: 245 ml Basal medium (from PSC Neural Induction Medium kit) + 245 ml advanced DMEM/F12 (ThermoFisher# 12634010) + 10 ml 50x supplement (from PSC Neural Induction Medium kit)

Rosette Selection Reagent (StemCell# 05832)

ROCK inhibitor (R&D Systems# 1254/1)

Accutase (StemCell# 07920)

Matrigel (Corning# 354277)

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- Zhang, H., Zhang, S., He, X. and Duan, J. (2021). 1.2. Differentiation of iPSCs into neural progenitor cells (NPCs). Bio-protocol Preprint. [bio-protocol.org/preprint/1271](https://www.bio-protocol.org/preprint/1271).
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M., Avramopoulos, D., Cowan, C. A., Chen, M., Pang, Z. P., Gejman, P. V., He, X. and Duan, J.(2020). Allele-specific open chromatin in human iPSC neurons elucidates functional disease variants . Science 369(6503). DOI: [10.1126/science.aay3983](https://doi.org/10.1126/science.aay3983)

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